

SUSCEPTIBILITY OF THAI ZOOPHILIC ANOPHELINES AND SUSPECTED MALARIA VECTORS TO LOCAL STRAINS OF HUMAN MALARIA PARASITES

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Abstract. Wild caught zoophilic *Anopheles* and suspected malaria vector species collected in northwest Thailand were experimentally infected with local human malaria parasites using a membrane feeding. One week post-feeding a number of mosquitos were dissected for oocyst examination. The remainder were kept for another one week or more, and then the salivary glands were examined for the presence of sporozoites. The results revealed that *An. vagus*, *An. kochi* and *An. annularis* were susceptible to both *Plasmodium falciparum* and *P. vivax* whereas *An. barbirostris* and *An. sinensis* were susceptible to only *P. vivax*. The non-susceptibility to *P. falciparum* of these two mosquito species may indicate their poor vector status of this malaria species in the field.

INTRODUCTION

To date at least 72 anopheline species have been recorded in Thailand (Harrison *et al*, 1990), but only *An. minimus s.l.*, *An. dirus s.l.* and *An. maculatus s.l.* are considered major malaria vectors with *An. sundai-cus* and *An. aconitus* as secondary vectors (Ketrangsee *et al*, 1991). More recently, *An. pseudowillmori* has been incriminated as a vector (Green *et al*, 1991). The role of other *Anopheles* species in malaria transmission is less clear. Several species have been suggested as suspected vectors, *eg. An. campestris*, *An. barbirostris*, *An. philippinensis/An. nivipes* and *An. culicifacies* (Prasittisuk, 1985) as they are the vectors in other Asian countries, but there is still no evidence of their vector status in Thailand.

The identification of a mosquito as a potential transmitter of malaria is dependent on the detection of sporozoite invasion of the salivary glands. Detection of *Plasmodium* sporozoites in mosquitos can be done by dissection of the salivary glands or detection of circumsporozoite (CS) antigens by an ELISA method (Burkot *et al*, 1984; Wirtz *et al*, 1985). The ELISA has been extensively used to determine sporozoite rates in the major vectors as well as to investigate other possible vectors throughout Thailand. Apart from the known vectors, at least 10 anopheline species in Thailand, have been reported positive for *P. falciparum* and/or *P.*

vivax CS antigens, *ie. An. barbirostris*, *An. sinensis*, *An. kochi*, *An. vagus*, *An. annularis*, *An. nivipes*, *An. peditaeniatus*, *An. tessellatus*, *An. nigerrimus* and *An. karwari* (Gingrich *et al*, 1986; Baker *et al*, 1987; Harbach *et al*, 1987; Gingrich *et al*, 1990). However, there were either no corresponding dissection data or no epidemiological studies to support their vector status. In addition, positive ELISA results do not necessarily mean that the mosquitos are infective since this method can detect CS protein from developing oocysts (Beier *et al*, 1987), soluble CS protein shed from oocysts and sporozoites (Verhave *et al*, 1988), and CS protein in various body parts (Robert *et al*, 1988). Some mosquito species develop heavy malaria infections but are not vectors since sporozoites do not invade salivary glands (Rosenberg, 1985). Moreover, since the zoophilic mosquitos were normally collected from or near bovines and often many of them were tested as whole body with blood (*eg* Gingrich *et al*, 1986; Gingrich, personal communication), the possibility of false positive results due to animal blood (Somboon *et al*, 1993) should not be ignored.

The importance of zoophilic anophelines in malaria transmission is likely to depend not only on their density, behavior trials and local ecological conditions, but also, of course, on their susceptibility to infection by local strains of human *Plasmodium*. So far, little is known about the susceptibility of zoophilic *Anopheles* species to human malaria parasites in Thailand. This was investigated in the present study.

MATERIALS AND METHODS

Wild caught, rather than laboratory reared, female mosquitos were used for this study as this consumes less time and man-power. In addition, local strains of human malaria parasites are easily available only in endemic areas, and taking gametocytemic blood to the laboratory for experimental feeding is difficult and results in a loss of infectivity (Somboon and Morakote, 1990). Experimental feeding was carried out at the field station.

Mosquito collections and handling

Unfed *Anopheles* mosquitos were collected from cattle corrals and human baits in the forest-fringe villages, Mae Sariang District, Mae Hong Son Province. In order to minimize handling prior to feeding and dissection, the collected mosquitos of mixed species were kept in cages provided with sugar solution. Twelve hours before feeding they were fasted and only cotton soaked with water offered.

Human malaria parasites

Blood slides of malaria patients presented at Mae Sariang Malaria Sector were examined. The blood of those patients with a gametocyte density over 10 per 100 wbc and who had acquired infection in the area (according to interview) was collected by using a heparinized syringe and transported to the field station.

Experimental feeding and dissection

The mosquitos and a known susceptible species were allowed to feed on the blood through parafilm membranes using blown glass feeders warmed at 37°C with a circulating water pump (Rutledge *et al*, 1964). Fully engorged membrane-fed females were maintained in the field station, in a humid chamber at ambient temperature (24-29°C), or they were transferred to the insectary (25-28°C, 70-80% RH) in Chiang Mai. On day 7 post-infection, a small number of them (usually 5) were identified to species and then dissected for oocyst examination. Further dissections were performed for the species which were found negative for oocyst, until either all had been dissected or one or more positive mosquitos were detected. The remainder were reared until day 15-17 when the salivary glands were examined for the presence of sporozoites

under a microscope. The midguts of salivary gland negative mosquitos were also examined for oocyst infection at this time. In order to confirm that any sporozoites found in these wild caught mosquitos were truly human malaria parasites, the sporozoites were tested by the ELISA technique (Burkot *et al*, 1984; Wirtz *et al*, 1985) to identify the malaria species. The glands were transferred into microcentrifuge tubes containing ELISA blocking buffer solution with the aid of needle under a dissecting microscope.

RESULTS

Although there was little problem in feeding mosquitos in the field with patients' blood through the membrane, a high mortality of fed mosquitos was observed in the second week after feeding. Somewhat low numbers of mosquitos were dissected for oocyst examination because the majority of them were kept to allow sporozoite development in salivary glands. Some species were collected in low numbers because of the season. The results are shown in Table 1. All four batches of *An. minimus* species A, a known susceptible species, were infected, confirming that the parasites were definitely infective. *An. vagus*, *An. kochi* and *An. annularis* were susceptible to both *P. falciparum* and *P. vivax*. In addition, susceptibility of *An. sawadwongporni* and *An. willmori* to *P. vivax* was demonstrated; these species were not tested against *P. falciparum*. It was interesting to note that all *P. falciparum* oocysts found in *An. sinensis* and *An. barbirostris* were degenerated whereas those of *P. vivax* normally developed. In some individuals of these two species, melanization in the degenerated oocysts and proliferation of hemocytes were observed on dissection following a *P. falciparum* infected blood meal.

Confirmation of positive salivary glands by the ELISA in all cases indicated the human malaria species. In all mosquitos which were negative for salivary gland sporozoites, no oocysts were observed on the midguts, confirming the absence of infection.

DISCUSSION

The apparent susceptibility of *An. sinensis* and *An. barbirostris* to *P. vivax*, but not to *P. falciparum*, may be due to local species or strain specific interactions and may not apply in other places. Although more studies are needed to confirm this, the present evidence

Table 1

Susceptibility of *Anopheles* mosquitos collected in Mae Sariang District, Mae Hong Son Province, to local strains of *Plasmodium falciparum* (PF) and *Plasmodium vivax* (PV) malaria parasites. The unfed female mosquitos were collected from cattle corrals and human baits and allowed to feed on blood of carriers via membrane feeding. There were two PF (PF1 and PF2) and two PV (PV1 and PV2) carriers.

<i>Anopheles</i>	Carrier no.	No. positive with oocyst/ no. dissected	Mean no. of oocyst (range)	No. positive with sporozoite*/ No. dissected
<i>Plasmodium falciparum</i>				
<i>An. vagus</i>	PF1	4/10	4.5 (1-9)	3/6
<i>An. kochi</i>	PF1	5/9	5.4 (1-17)	2/3
	PF2	5/7	25.4 (5-56)	4/6
<i>An. annularis</i>	PF1	1/3	2.0 (2)	1/3
	PF2	4/9	23.0 (16-35)	5/6
<i>An. minimus A</i>	PF1	3/4	9.0 (3-15)	2/2
	PF2	4/5	31.0 (15-48)	3/4
<i>An. sinensis</i>	PF2	0/29	-	-
<i>An. barbirostris</i>	PF2	0/16	-	-
<i>Plasmodium vivax</i>				
<i>An. vagus</i>	PV1	5/5	6.6 (3-11)	3/3
<i>An. kochi</i>	PV1	1/1	47.0 (47)	NA
	PV2	5/6	59.8 (2-135)	2/3
<i>An. annularis</i>	PV1	4/4	9.3 (2-30)	NA
	PV2	3/3	45.0 (21-65)	5/7
<i>An. minimus A</i>	PV1	2/3	10.0 (9-11)	1/1
	PV2	2/2	32.0 (25-39)	3/3
<i>An. sawadwongporni</i>	PV1	1/1	12 (12)	2/2
<i>An. willmori</i>	PV1	1/2	5 (5)	1/1
<i>An. sinensis</i>	PV2	5/6	28.2 (1-101)	8/13
<i>An. barbirostris</i>	PV1	1/1	7 (7)	NA
	PV2	4/5	12.3 (5-22)	2/3

* In salivary glands; NA, Not available

for non-susceptibility is reasonably strong: a large proportion of females of other species were infected by the same blood at the same time. Experimental infections performed in south China showed that *An. sinensis* was negative for *P. falciparum*, while the stomach wall of the closely related species *An. lesteri* (= *An. anthropophagus*) was occasionally infected with young oocysts, indicating some degree of susceptibility of this species to *P. falciparum* (Otsuru and Ohmori, 1960; Beales, 1984). Low susceptibility to *P. falciparum* (0-14.3% oocyst rate and 0-9% sporozoite rate) of the *An. sinensis* group in China was confirmed by Zheng *et al* (1989). Recently, Baimai *et al* (1993) reported that there are two karyotypic forms of *An. sinensis* in Thailand, but whether they differ in susceptibility to human malaria or vectorial capacity is not known.

Little is known about the susceptibility of *An. barbirostris* to human malaria parasites, but it has a low susceptibility to *P. cynomolgi bastianelli* (Warren *et al*, 1963). It has been found to be infected in nature with *Plasmodium* species in many Asian countries (Rao, 1984); however, since the recognition of a sibling group in this species (Reid, 1962), some of the older reports are now regarded as concerning two closely related species, ie *An. campestris* and *An. donaldi*. These two species are anthropophilic and endophagic and are important vectors of malaria in Malaysia (Reid, 1968). *An. barbirostris* is not regarded as a malaria vector, except perhaps in the Sulawesi (Celebes) Islands, Indonesia (Reid, 1968). *An. barbirostris* and *An. sinensis* are widely distributed throughout Thailand (Harrison and Scanlon, 1975). The present evidence suggests that these two species may not be suitable hosts for *P. falciparum*, at least in northwest Thailand. There should be investigations to see whether they are also refractory to *P. falciparum* in other regions of the country.

The present study also suggests that many species of zoophilic anopheline mosquitos in Thailand are susceptible to human malaria parasites, although some may not be susceptible to *P. falciparum*. Some species are known to be the vectors of malaria in neighboring countries, eg *An. annularis* in Nepal and Myanmar, *An. willmori* in Nepal, *An. sinensis* in China, *An. barbirostris* in Indonesia (Rao, 1984). Although they normally prefer animal blood, all have been caught frequently in human-biting collections and may be important in malaria transmission as investigated by Gould *et al* (1967) and Green *et al* (1991). The demonstration of susceptibility does not establish the importance of a given species as a vector in the field, but consistent

demonstrations of refractoriness can be useful to rule out this possibility.

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